REMARKS

The Examiner objected to the title of the abstract and the title of the invention. The title and abstract have been amended in accordance with the Examiner's comments, and Applicants respectfully submit that the title and abstract are descriptive of the invention.

The Examiner also objected to the citation of reference FR 2 695 392 on the Information Disclosure Statement filed February 12, 2002, as failing to comply with 37 CFR 1.98(a)(3) because it did not include a concise explanation of the relevance of the patent. Applicants are submitting herewith an IDS listing the reference and also a copy of an English translation of the abstract of this reference. Applicants respectfully submit that the submission of this translated abstract fulfills the requirements of 37 CFR 1.98(a)(3) and that the reference should be considered.

Claims 1-8, 12-16, and 22 are pending in the application. Claims 3 and 4 have been cancelled. Claims 1, 2, 14, and 22 have been amended. New claim 23 has been added. The amended and newly added claims emphasize the importance to the invention that the nucleotide sequences of the claims share a high percentage of identity to the exemplary defensin nucleotide sequence set forth in SEQ ID NO:4. Support for newly added claim 23 can be found in the specification, particularly on pages 2, 8, 14, and 18. No new matter has been added by way of amendment. Reexamination and reconsideration of the claims are respectfully requested.

The Rejection of Claims Under 35 U.S.C.§112, First Paragraph, Should Be Withdrawn

The Office Action (11/21/02, page 3, #8) has rejected claims 1-8, 12-16, and 22 under 35 U.S.C. §112, first paragraph, as failing to meet the enablement requirement. Claims 3 and 4 have been cancelled, and independent claim 1 (and thereby the remaining claims 1-8, 12-16, and 22, which are dependent on or incorporate the limitations of claim 1) has been amended to recite that the nucleotide sequence encodes a polypeptide that has an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:4. Applicants also note that independent claim 1 requires that the polypeptide have defensin activity. Support for these

amendments can be found in the original claims and in the specification, more particularly, for example, on pages 2, 14, and 18. While the rejection has not been raised against the claims as amended, the rejection will be addressed in so far as it may apply to the amended claims.

The Office Action (page 3) asserts that "[t]he claims are broadly drawn to a multitude of nucleic acids..." but that "[t]he instant specification... only provides guidance for construction of cDNA libraries from *Vaejovis carolinianus* (Kentucky scorpion), *Scolopendra canidens* (centipede), and *Argiope* spp. (orb spider) and random sequencing of the clones (example 1); identification of cDNAs as potentially encoding defensins by BLAST comparison to sequence databases and identification of SEQ ID NO:3 from *V. carolinianus* as encoding a protein (SEQ ID NO:4) with 75.7% identity to a defensin from *Androctonus australis hector* (examples 2-3), and general guidance for expression of genes in monocots, dicots and microbes (examples 4-6)." The Office Action concludes that "[t]he instant specification fails to provide guidance for isolation or construction of nucleic acids encoding proteins with defensin activity...." Applicants respectfully disagree with this assessment.

The present specification provides full-length nucleotide and amino acid sequences of defensins from *Scolopendra canidens* (SEQ ID NOs: 1 and 2, respectively), *Vaejovis carolinianus* (SEQ ID NOs: 3 and 4), and *Argiope* spp. (nucleotide sequences set forth in SEQ ID NOs: 5, 7, and 9; amino acid sequences set forth in SEQ ID NOs: 6, 8, and 10, respectively). See particularly Table 1 on page 5 of the specification. In this manner, the present specification provides an array of novel defensins from various arthropods.

As the Office Action acknowledges, the *Vaejovis carolinianus* defensin (SEQ ID NO:4) shares 75.7% identity to a previously-identified defensin from the scorpion *Androctonus australis hector* (Office Action at p. 3). However, the Office Action states that "SEQ ID NO:4 has ***only*** 75.7% identity to the defensin from *Androctonus australis hector*" (emphasis added) and concludes that "[t]he specification provides no evidence that SEQ ID NO:4 actually encodes a protein with defensin activity." (Office Action at p. 4).

Applicants respectfully disagree with this conclusion. In fact, Applicants have provided art-accepted methods to establish that the *Vaejovis carolinianus* polypeptide of SEQ ID NO:4 is

a defensin as outlined in detail below. First, the polypeptide of SEQ ID NO:4 is cationic, with a predicted pI of 9.44 (see Appendix C, analysis of SEQ ID NO:4 using ExPASy software). Further, the polypeptide of SEQ ID NO:4 contains the conserved cysteine residues found in insect defensins. These conserved cysteine residues are well-known in the art. For example, Bulet *et al.* (1999) (*Dev. Comp. Immunol.* 23: 329-344) discusses the "striking feature that emerges from the comparison of the three-dimensional structures of insect defensins,...a motif named "Cysteine Stabilized α-helix (CSH). This scaffold refers to an invariant Cys-Xaa-Cys sequence found in a strand of a β-sheet in which two cysteine residues are attached to two cysteine residues of the stretch Cys-Xaa-Xaa-Xaa-Cys present in an α-helix...." (pp. 334-335). A diagram of these common motifs of cysteine residues in a multiple alignment of insect and scorpion defensins is shown in, for example, Cho *et al.* (1995) (*Insect Biochem. Mol. Biol.* 26: 395-402) (p. 399, Figure 2; see also Appendix A, which reproduces Figure 1 of the present specification above a portion of Cho's Figure 2; dashed lines indicate conserved cysteines also highlighted by Cho; highlighted residues are conserved cysteines). Applicants note that the exemplary disclosed sequence of SEQ ID NO:4 contains all six of these cysteine residues.

The *V. carolinianus* polypeptide of SEQ ID NO:4 has also been compared to the Pfam database of protein families and been shown to share a high degree of sequence similarity with the consensus structure for Arthropod defensins (PFAM Accession No. PF01097; see Appendix B). The Arthropod defensin family is described in the Pfam database as "a family of insect and scorpion cysteine-rich antibacterial peptides, primarily active against Gram-positive bacteria (see Appendix B). The Pfam database provides a curated collection of well-characterized protein family domains with high quality alignments. Functional domains of novel proteins may be identified by comparison with the Pfam protein family domain alignments. It is well known in the art that regions of sequence homology with known functional domains may be used to determine protein function. Accordingly, the presence of a Pfam consensus structure for the Arthropod defensin family in the *V. carolinianus* sequence of SEQ ID NO:4 indicates that this polypeptide functions as a defensin.

Furthermore, the United States Patent and Trademark Office accepts sequence homology as a basis for establishing utility. While here the rejection is characterized as being an enablement rejection under 35 U.S.C. §112, first paragraph, such rejections are typically made under both 35 U.S.C. §101 and 35 U.S.C. §112. The rationale for such rejections is that a sequence has no utility if it has no known function and therefore one of skill in the art would not know how to use it. Here, the rejection has been made only under 35 U.S.C. ss 112, first paragraph, but the rejection hinges on whether "[t]he specification provides no evidence that SEQ ID NO:4 actually encodes a protein with defensin activity." (Office Action, page 4). The United States Patent and Trademark Office "Utility Examination Guidelines" (66 Fed Reg. 1092 (2001)) make it clear that sequence homology is sufficient to establish utility, and that, contrary to the standard being applied here, working examples or biochemical evidence are not a *per se* requirement for the establishment of utility. The "Utility Examination Guidelines" state, "[w]hen a patent application claiming a nucleic acid asserts a specific, substantial, and credible utility, and bases the assertion upon homology to existing nucleic acids or proteins having an accepted utility, the asserted utility must be accepted by the examiner unless the Office has sufficient evidence or sound scientific reasoning to rebut such an assertion" (66 Fed. Reg. 1096). In the present case, the Examiner has not accepted the asserted utility for the claimed invention but has failed to provide sufficient evidence or sound scientific reasoning to rebut Applicants' assertions.

Of the examples given in the utility guideline training materials, Example 10, which is directed to a sequence that has sequence similarity with a DNA ligase, is most analogous to the present application. As in Example 10, the *V. carolinianus* defensin has been shown to share sequence similarity with a protein family of known function. As in Example 10, the protein family of known function has a well-established utility, as discussed in more detail elsewhere herein. Accordingly, based on analogy to Example 10, the present invention also meets the criteria for well-established utility and therefore should also be deemed to be enabled.

The sequence of Example 10 is accorded to have a specific and substantial utility according to the "Revised Interim Utility Guidelines Training Material Examples," *ibid.*, despite

the fact that the encoded polypeptide has not been directly demonstrated to have DNA ligase activity, and the substrate (*i.e.*, single-stranded DNA or double-stranded DNA, blunt-ended DNA, 5′ recessed ended DNA, 3′ recessed ended DNA), co-factor requirements, and reaction conditions required to practice the invention are not disclosed. Thus, in accordance with the Utility Examination Guidelines, the very fact that the sequence of Example 10 has sequence similarity with a known protein possessing well-established utility is sufficient to confer a specific, substantial, and credible utility upon the claimed sequence. As the claimed invention of the present application is analogous to the situation described in Example 10, the criteria for utility have been met by the present claims.

Furthermore, those of skill in the art recognize that sequence homology is a predictor of protein function. The prior art is replete with references discussing the defensin gene families from a wide array of organisms. Defensins are described by those of skill in the art as genes which contain the characteristic conserved cysteines, and defensin gene families are described as sharing properties of relatively short length and amino acid similarities from 58% to 95%. See, *e.g.*, Bulet *et al.* (1999) *Dev. Comp. Immunol.* 23: 329-344 at pp. 330-331; Cho *et al.* (1995) *Insect Biochem. Mol. Biol.* 26: 395-402; White *et al.* (1995) *Curr. Op. Struct. Biol.* 5: 521-527; Broekaert *et al.* (1995) *Plant Physiol.* 108: 1353-1358.

The assays that have been performed on proteins having these properties have confirmed that such proteins have defensin activity. See, *e.g.*, Lamberty *et al.* (1999) *J. Biol. Chem.* 274: 9320-9326; Thevissen *et al.* (1999) *App. Env. Microbiol.* 65: 5451-5458; Terras *et al.* (1995) *Plant Cell* 7: 573-588. The examples given here, which demonstrate the accuracy of sequence similarity-based determinations of protein function, represent only a few of the many such instances that are found in the scientific literature. Because of evidence such as that presented above, methods of using sequence homology within the functional domains of proteins of known function to determine the function of novel proteins have become widely accepted by those of skill in the art as reliable and accurate. Accordingly, one of skill in the art would readily accept that the *V. carolinianus* protein of SEQ ID NO:4 functions as a defensin based on the types of evidence presented in the specification. Because one of skill would accept that the exemplary

disclosed sequences are defensins, the rejection of the claims under 35 U.S.C. §112, first paragraph, for lack of enablement on the basis that the disclosed sequences are not defensins should be withdrawn.

The Office Action states a further basis for the enablement rejection on page 5, concluding that "[e]xpressing pesticidal peptides in plants is unpredictable," and that "[p]eptides that are effective pesticides when isolated and fed to insects do not function as pesticides when genes encoding them are transformed into plants" (p. 5). The Office Action cites for support Pang *et al.* (1992), characterizing this reference as teaching that tobacco plants transformed with a gene encoding the scorpion insectotoxin I_5A had no paralytic effect on tobacco budworm. The Office Action also cites for support Barton *et al.* (1993), characterizing this reference as teaching that a neurotoxin from *Androctonus australis hector* was not effective against tobacco hornworm when expressed in plants even though it was effective when topically applied to the hornworms.

However, there are several critical differences between the present invention and the cited references which render these references essentially irrelevant to the present claims. First, both of the cited studies sought to control large insect predators through expression of proteins. In contrast, defensins are known in the art to provide a wide array of protection against many species of pathogens, primarily Gram-positive bacteria. See, for example, Boman (1995) *Ann. Rev. Immunol.* 13: 61-92, which reviews the groups of known "peptide antibiotics" and notes that defensins act on a wide variety of bacteria and fungi but act more effectively on Gram-positive bacteria than they do on Gram-negative bacteria (p. 70). See also, White *et al.* (1995) *Curr. Op. Struct. Biol.* 5: 521-527, which discusses that the insect defensin category, which includes the defensin from the scorpion *Leiurus quinquestriatus*, has an antimicrobial spectrum that primarily encompasses Gram-positive bacteria (p. 522, Table 1). Cho *et al.* (1996) *Insect Biochem. Mol. Biol.* 26: 395-402 teaches that while insect defensins are mostly effective against Gram-positive bacteria, it has been reported that at least one Gram-negative bacterium was susceptible to mosquito defensin and therefore other Gram-negative bacteria may also be sensitive to insect defensins. Similarly, Bulet *et al.* (1999) *Dev. Comp. Immunol.* 23: 329-344

teaches that insect defensin-like peptides have been found in the scorpions *Leiurus quinquestriatus* and *Androctonus australis hector*[1] (pp. 330-331) and are active against a wide range of Gram-positive bacteria but only against a few examples of Gram-negative bacteria, fungi and yeasts. (p. 332, col. 1). See also, Hetru *et al.* (1998) "Antimicrobial peptides from insects," in *Molecular Mechanisms of Immune Responses in Insects,* ed. Brey and Hultmark (Chapman & Hall, London), particularly pp. 45-49. The Bulet reference continues, "[n]umerous studies conducted on various native insect defensins established that these peptides have an almost immediate lytic effect on the Gram-positive bacteria." (p. 332, col. 2).

While the defensins of the present invention may additionally provide protection of transformed plants against insect pathogens, some defensins are well-known in the art to provide protection against bacterial and fungal infection. See, *e.g.*, Terras *et al.* (1995) (*Plant Cell* 7:573-588), cited on page 1 of the present specification, which teaches that transgenic tobacco expressing a radish defensin were up to eightfold more resistant to lesion formation by tobacco foliar pathogen *A. longipes* than comparable nontransgenic plants (p. 579, col. 2). Brockaert *et al.* (1995) *Plant Physiol.* 108: 1353-1358 teaches that "several members of the plant defensin family inhibit growth of a broad range of fungi at micromolar concentrations." (pp. 1354-1355). Plant defensins that exert antifungal effects fall into two categories, "morphogenic" and "nonmorphogenic." Morphogenic plant defensins decrease hyphal elongation and increase hyphal branching, while nonmorphogenic plant defensins decrease the rate of hyphal extension. (p. 1355, col. 1).

Thus, the defensins of the present invention, while they may have additional antibiotic activities, are most expected to be effective against bacterial pathogens. For this reason, the fact that the Pang and Barton studies could not control insects using proteins that were not defensins is essentially irrelevant to the present invention. As discussed above, numerous studies have demonstrated the efficacy of defensins against a wide array of bacterial and fungal pathogens.

[1] The *Leiurus quinquestriatus* defensin is 94% identical to the *Androctonus australis hector* defensin sequence shown in the alignment in Figure 1 of the present specification, differing at only 2 amino acid positions (see Ehret-Sabatier *et al.* (1996) *J. Biol. Chem.* 271:29537-29544, particularly Figure 2 on page 29540).

Therefore the rejection of the claims on the basis of the unrelated failures of Pang and Barton should be withdrawn.

The Office Action concludes that an undue amount of experimentation would be required to make a nucleotide sequence that encodes a polypeptide which is at least 80% identical to SEQ ID NO:4. Applicants note that independent claim 1 (and hence claims 1-8, 12-16, and 22, which are dependent on or incorporate the limitations of claim 1) has been amended to recite that the nucleotide sequence encodes a polypeptide that has an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:4. Applicants note that independent claim 1 also requires that the polypeptide have defensin activity.

Applicants believe that those of skill in the art, provided the guidance in the present specification, could readily make and use the claimed invention. Guidance for determining percent identity of sequences is provided in the specification, for example, on pages 8-9. Those of skill in the art can readily determine the nucleic acid sequence of a nucleic acid molecule as well as the percent identity between sequences.

Moreover, claim 1 specifies that the polypeptide of the method has defensin activity and therefore encompasses functional variants. Defensins are well-known in the art and are known to provide protection to transgenic plants (see specification, *e.g.*, on pp. 1, 2 and 16). Assays and procedures are known in the art to readily determine whether a sequence has defensin activity (see specification pp. 16-17). For example, Terras *et al.* (1995) *Plant Cell* 7:573-588 teaches an assay for defensin antifungal activity from germinating plant seeds (p. 574, col. 1 and Figure 1) as well as tissue-print immunolocalization assays for monitoring expression of defensins in various plant parts (p. 575, col. 2), assays for the enhanced disease tolerance of transgenic plants expressing defensins (p. 578, col. 2-p.580), and assays of *in vitro* defensin activity from transgenic plants (p. 580, col. 2). Other references teach assays for expression of particular genes; for example, Oh *et al.* (1999) *Plant Mol. Biol.* 41:313-319 teaches assays for determining whether the expression of particular genes has been induced by various treatments. Thevissen *et al.* (1996) *J. Biol. Chem.* 271: 15018-15025 teaches assays for antifungal activities of defensins, and Thevissen *et al.* (1999) *App. Env.*

Microbiol. 65: 5451-5458 teaches an assay for permeabilization of fungal membranes by defensins. Lamberty *et al.* (1999) *J. Biol. Chem.* 274: 9320-9326 teaches assays for antifungal and antibacterial activities of defensins.

Thus, in contrast to the Examiner's conclusions, guidance is provided as to which region of the sequence of SEQ ID NO:4 can be altered and still provide a polypeptide species encompassed by the claim. Applicant has provided the exemplary nucleotide sequence of SEQ ID NO:3 and the exemplary amino acid sequence of SEQ ID NO:4. The claimed sequences of the invention vary from this sequence by structural parameters (*i.e.*, percent sequence identity to SEQ ID NO:4, or encoding such a polypeptide) and are required to retain defensin activity.

Thus, a rational scheme for determining the regions of the defensin polypeptides encoded by the claimed sequence that would tolerate modification is provided. Based on the exemplary defensin nucleotide and polypeptide sequences provided and the known methods for identifying additional residues critical for defensin activity, the skilled artisan could choose among possible modifications to produce polypeptides within the parameters set forth in the claims and then test these modified variants to determine if they retain defensin activity.

Applicants believe that undue experimentation would not be required for one of skill in the art to make and use the claimed invention. The standards for assessing whether undue experimentation is necessary have been discussed in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and *In re Jackson*, 217 USPQ 804, 807 (Bd. Pat. App. & Int. 1982). In *In re Jackson*, the Board held that a considerable amount of experimentation is permitted to practice the invention and is not undue if it is merely routine in the art or if the specification provides a reasonable amount of guidance and direction to perform such experimentation. Applicants note that it is now customary in the art to make and assay a number of sequences for a desired function in order to achieve the best results. For example, such techniques can involve what is commonly referred to as "shuffling," as described for example in U.S. Patent No. 5,837,458, issued November 17, 1998 with inventors Minshull and Stemmer and entitled, "Methods and Compositions for Metabolic and Cellular Engineering." In such techniques, it is common to mutagenize individual sequences or a set of sequences which are then assayed for a desired

activity. In fact, such techniques may even make use of a library of sequences which is recursively mutagenized, screened for function using a functional assay, and re-mutagenized. Examples of the use of such techniques include: Minshull and Stemmer (1999) *Current Opinion in Chemical Biology* 3:284-290, entitled "Protein Evolution by Molecular Breeding"; and Christians *et al.* (1999) *Nature Biotechnology* 17: 259-264, entitled "Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling." Such experiments are designed and are intended to encompass the generation and testing of a very large number of variant sequences for a desired function. As indicated by these and other publications, this experimentation is now considered routine in the art and thus would not be considered "undue experimentation" under *In re Wands* and *In re Jackson*.

Applicants stress that when evaluating the quantity of experimentation required, the court looks to the amount of experimentation required to practice a single embodiment of the invention, rather than the amount required to practice every embodiment of the invention. For example, in *Wands*, the claims at issue were drawn to immunoassay methods using any monoclonal antibody having a binding affinity for HbsAg of at least 10^{-9} M. The PTO had taken the position that the claim was not enabled as it would take undue experimentation to make the monoclonal antibodies required for the assay. The Federal Circuit reversed and held that the claims were enabled, as the amount of experimentation required to isolate monoclonal antibodies and screen for those having the correct affinity was not undue. *See Id.* Clearly, the Federal Circuit did not contemplate that every antibody useful in the methods of the claim must be identified. Rather, the court considered the amount of experimentation required to identify one or a few monoclonal antibodies having the required affinity. *See also, Johns Hopkins University v. Cellpro*, 931 F. Supp. 303, 324 (D. Del. 1996), *aff'd in part, vacated in part, and remanded*, 47 USPQ2d 1705 (Fed. Cir. 1998) (stating that "[t]he specification need only enable one mode of making the claimed invention.").

Here, the practice of the invention requires essentially two steps: generating a polynucleotide having a sequence that meets the applicable limitations of the claims and assaying the encoded polypeptide for defensin activity. Guidance for performing these steps is

provided in the specification and well-known in the art. The additional embodiments of the dependent claims incorporate further limitations which are also taught in the specification and readily created by those of skill in the art. Ample guidance is therefore provided to allow one of skill in the art to identify additional sequences encompassed by the claims. Consequently, the quantity of experimentation necessary and the amount of guidance presented in the specification is sufficient to enable the claimed polynucleotides and their methods of use as set forth in the claims. Therefore, Applicants respectfully request that the rejection of the claims under 35 U.S.C. §112, first paragraph, be withdrawn and not applied to the amended claims.

The Office Action (11/21/02, page 6, #9) has rejected claims 1-8, 12-16, and 22 under 35 U.S.C. §112, first paragraph, "as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." While the rejection has not been raised against the claims as amended, the rejection will be addressed in so far as it may apply to the amended claims.

Independent claim 1 (and thereby claims 1-8, 12-16, and 22, which are dependent on or incorporate the limitations of claim 1) has been amended to recite that the nucleotide sequence encodes a polypeptide that has an amino acid sequence that is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:4. Applicants also note that independent claim 1 requires that the polypeptide have defensin activity. Support for these amendments can be found in the original claims and in the specification, more particularly, for example, on pages 2, 14, and 18.

The Office Action states that the written description of the subject matter of the claims is not adequate to demonstrate that Applicants were in possession of the claimed invention at the time this application was filed because the specification does not provide sufficient characteristics of the claimed variants. Applicants respectfully disagree.

Amended independent claim 1 recites that the polypeptide encoded by the claimed polynucleotide has at least 90% identity to the sequence set forth in SEQ ID NO:4. The recitation of at least 90% sequence identity is a *very predictable structure* of the sequences

encompassed by the claimed invention. Applicants note that the description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. 66 Fed. Reg. 1099, 1106 (2001). Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. 66 Fed. Reg. 1099, 1106 (2001). Applicants submit that the knowledge and level of skill in the art would allow a person of ordinary skill to envision the claimed invention, *i.e.*, a nucleotide sequence encoding a polypeptide having at least 90% sequence identity to the sequence set forth in SEQ ID NO:4.

Furthermore, the description of a claimed genus can be by structure, formula, chemical name, or physical properties. *See, Ex parte Maizel*, 27 USPQ2d 1662, 1669 (B.P.A.I. 1992), citing *Amgen v. Chugai*, 927 F.2d 1200, 1206 (Fed. Cir. 1991). A genus of DNAs may therefore be described by means of a recitation of a representative number of DNAs defined by nucleotide sequence and falling within the scope of the genus, *or* by means of a recitation of structural features common to the genus, which features constitute a substantial portion of the genus. *See*, *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1569 (Fed. Cir. 1997); *see also* Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, First Paragraph, "Written Description" Requirement, 66 Fed. Reg. 1099, 1106 (2001). The recitation of a predictable polypeptide structure of at least 90% sequence identity to SEQ ID NO:4 is sufficient to satisfy the written description requirement.

An Applicant, however, may also rely upon functional characteristics in the description, provided there is a correlation between the function and structure of the claimed invention. *See Id.*, citing *Lilly* at 1568. Indeed, independent claim 1 recites functional characteristics of the claimed genus. Specifically, claim 1 recites that the claimed sequences further encode a polypeptide that has defensin activity; thereby providing a functional characterization of the sequences claimed in the genus. As discussed in more detail above and as indicated in the

specification (see, *e.g.*, pp. 16-17), those of skill in the art are familiar with assays to determine whether a particular polypeptide has defensin activity.

Example 14 of the "Synopsis of Application of Written Description Guidelines" is directed to a generic claim: a protein having at least 95% sequence identity to the sequence of SEQ ID NO:3, wherein the sequence catalyzes the reaction A → B. The synopsis materials conclude that the generic claim of Example 14 is sufficiently described under § 112, first paragraph, because: 1) "the single sequence disclosed in SEQ ID NO:3 is representative of the genus"; and 2) the claim recites a limitation requiring the compound to catalyze the reaction from A → B. The synopsis materials conclude that one of skill in art would recognize that the Applicants were in possession of the necessary common attributes possessed by the members of the genus.

Following the analysis of Example 14, Applicants submit that the present claims satisfy the written description requirements of § 112, first paragraph. Specifically, the claims of the present invention encompass a polynucleotide having a sequence encoding a polypeptide having at least 90% sequence identity to SEQ ID NO:4. As in Example 14, the specification discloses the nucleic acid sequence of SEQ ID NO:3 and the amino acid sequence of SEQ ID NO:4 and independent claim 1 recites a limitation requiring the compound to have a specific function (*i.e.*, defensin activity). Consequently, contrary to the conclusion in the Office Action, the sequences encompassed by genus claim 1 are defined by relevant identifying physical and chemical properties. In fact, the common attributes or features of the elements possessed by the members of the genus is that they encode polypeptides having defensin activity and sharing at least 90% sequence identity to the amino acid sequence of SEQ ID NO:4. Thus, the necessary common features of the claimed genus are clear.

The Office Action also cites for support of this rejection the Federal Circuit cases *Eli Lilly* and *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991); *cert. denied* 112 S.Ct. 169 (1991). However, both of these cases are incorrectly applied to the present situation. Applicants contend that the present claims and specification meet the 35 U.S.C. §112 written description requirement as clarified by *Eli Lilly* and *Amgen*. Applicants

have provided exemplary sequences of the invention as set forth in SEQ ID NOs:3 and 4. Applicants have thus provided a structural definition of the sequences of the invention. In addition, because defensins are well-known in the art, those of skill in the art can readily assess whether a nucleic acid molecule meeting the sequence element of the claims also meets the functional limitation element of the claims. This is what *Eli Lilly* requires. Thus, Applicants have also conceived the sequences of the invention as articulated in *Amgen*; that is, Applicants are able "to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it." *Amgen*, 18 USPQ2d at 1021.

In summary, the description of a representative number of species *does not* require the description to be of such specificity that it would provide individual support for each species that the genus embraces. Applicants submit that the relevant identifying physical and chemical properties of the disclosed genus would be clearly recognized by one of skill in the art and consequently, the Applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus. Accordingly, the rejection of claims 1-8, 12-16, and 22 under 35 U.S.C. §112, first paragraph, for lack of written description should be withdrawn and not applied to the newly submitted claims.

The Rejection of Claims Under 35 U.S.C.§112, Second Paragraph, Should Be Withdrawn

The Office Action (11/21/02, page 7, #11) has rejected claims 14 and 22 as being indefinite due to defects in antecedent basis. These claims have been amended to address these rejections, and Applicants respectfully submit that these rejections have been obviated by amendment.

CONCLUSION

In view of the above amendments and remarks, Applicants submit that the rejections of the claims under 35 U.S.C. §112, first and second paragraphs, are overcome. Applicants

respectfully submit that this application is now in condition for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject Application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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Lynda-Jo Pixley

RTA2132061v1

Version with Markings to Show Changes Made:

In the Specification:

Please revise the Title on page 1, line 2 to read as follows:

ARTHROPOD DEFENSINS OF *SCOLOPENDRA CANIDENS, VAEJOVIS CAROLINIANUS,* AND *ARGIOPE* SPP.

Please revise the Abstract on page 1, line 2 to read as follows:

This invention relates to an isolated nucleic acid fragment encoding a defensin which shares a high degree of sequence identity with the disclosed sequences from *Scolopendra canidens, Vaejovis carolinianus,* and *Argiope* spp. The invention also relates to the construction of a chimeric gene encoding all or a portion of the defensin, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the defensin in a transformed host cell.

In the Claims:

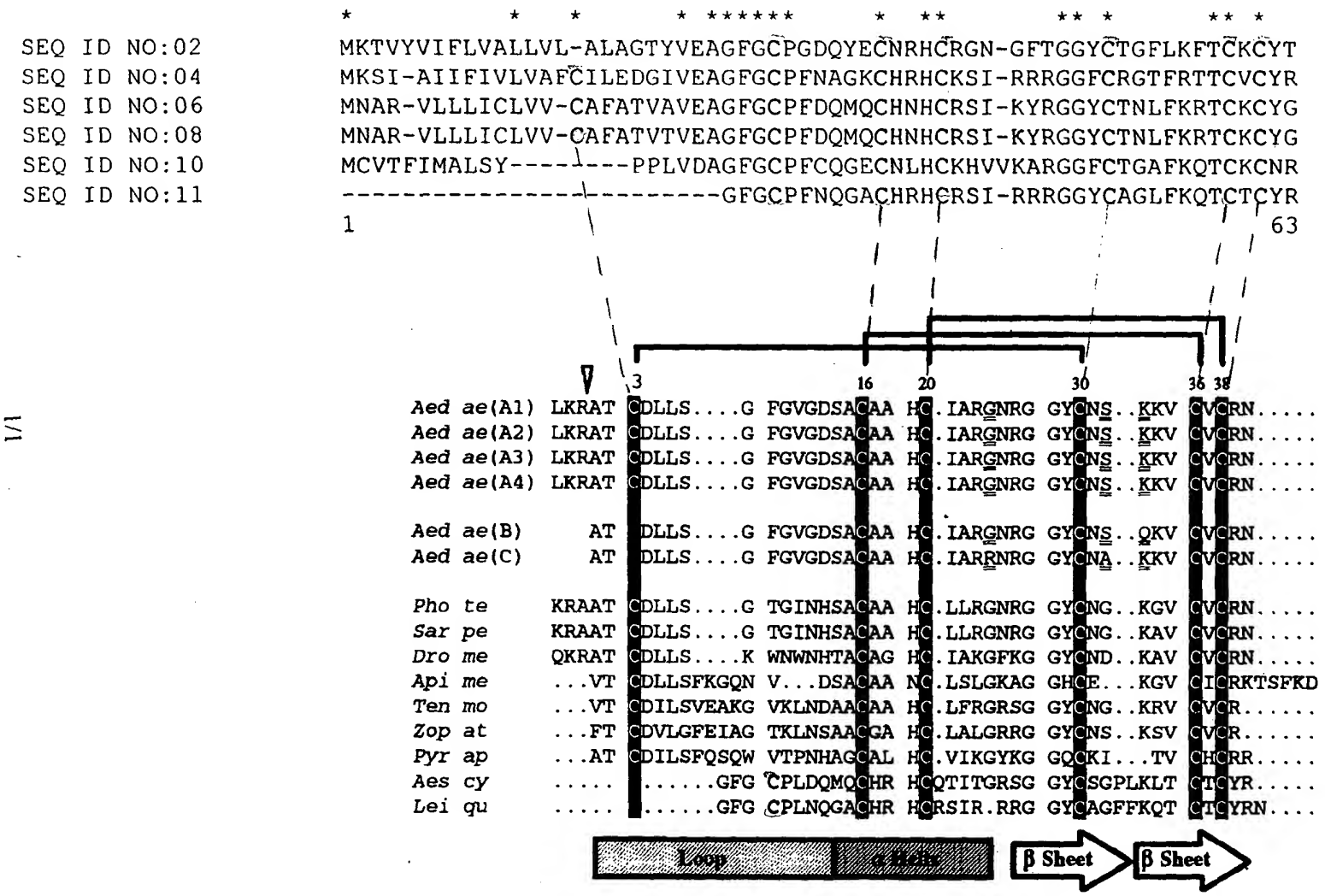
1. (Twice amended) An isolated polynucleotide comprising:
   (a) a nucleotide sequence encoding a polypeptide having defensin activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least [80%] 90% sequence identity [based on the Clustal alignment method], or
   (b) the complement of the nucleotide sequence.

2. (Twice amended) The isolated polynucleotide of Claim 1, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NO:4 have at least [85%] 95% sequence identity [based on the Clustal alignment method].

14. (Amended) A method for producing a transgenic plant comprising transforming a plant cell with the polynucleotide of Claim 1 to produce a transformed plant cell and regenerating a plant from [the] said transformed plant cell.

22. (Amended) A method for production of a polypeptide having defensin activity comprising the steps of cultivating the cell of Claim 13 in culture medium under conditions that allow for the synthesis of the polypeptide and isolating the polypeptide from said cell [the cultivated cells], from the culture medium, or from both [the cultivated] said cells and [the] said culture medium.

FIGURE 1


SEQ ID NO:02 MKTVYVIFLVALLVL-ALAGTYVEAGFGCPGDQYECNRHCRGN-GFTGGYCTGFLKFTCKCYT
SEQ ID NO:04 MKSI-AIIFIVLVAFCILEDGIVEAGFGCPFNAGKCHRHCKSI-RRRGGFCRGTFRTTCVCYR
SEQ ID NO:06 MNAR-VLLLICLVV-CAFATVAVEAGFGCPFDQMQCHNHCRSI-KYRGGYCTNLFKRTCKCYG
SEQ ID NO:08 MNAR-VLLLICLVV-CAFATVTVEAGFGCPFDQMQCHNHCRSI-KYRGGYCTNLFKRTCKCYG
SEQ ID NO:10 MCVTFIMALSY----PPLVDAGFGCPFCQGECNLHCKHVVKARGGFCTGAFKQTCKCNR
SEQ ID NO:11 GFGCPFNQGACHRHCRSI-RRRGGYCAGLFKQTCTCYR
1
63
3
16
20
30
36
38
Aed ae(A1) LKRAT CDLLS....G FGVGDSACAA HC.IARGNRG GYCNS..KKV CVCRN.....
Aed ae(A2) LKRAT CDLLS....G FGVGDSACAA HC.IARGNRG GYCNS..KKV CVCRN.....
Aed ae(A3) LKRAT CDLLS....G FGVGDSACAA HC.IARGNRG GYCNS..KKV CVCRN.....
Aed ae(A4) LKRAT CDLLS....G FGVGDSACAA HC.IARGNRG GYCNS..KKV CVCRN.....
Aed ae(B) AT DLLS....G FGVGDSACAA HC.IARGNRG GYCNS..QKV CVCRN.....
Aed ae(C) AT DLLS....G FGVGDSACAA HC.IARRNRG GYCNA..KKV CVCRN.....
Pho te KRAAT CDLLS....G TGINHSACAA HC.LLRGNRG GYCNG..KGV CVCRN.....
Sar pe KRAAT CDLLS....G TGINHSACAA HC.LLRGNRG GYCNG..KAV CVCRN.....
Dro me QKRAT CDLLS....K WNWNHTACAG HC.IAKGFKG GYCND..KAV CVCRN.....
Api me ...VT CDLLSFKGQN V...DSACAA NC.LSLGKAG GHCE...KGV CICRKTSFKD
Ten mo ...VT CDILSVEAKG VKLNDAACAA HC.LFRGRSG GYCNG..KRV CVCR.....
Zop at ...FT CDVLGFEIAG TKLNSAACGA HC.LALGRRG GYCNS..KSV CVCR.....
Pyr ap ...AT CDILSFQSQW VTPNHAGCAL HC.VIKGYKG GQCKI...TV CHCRR.....
Aes cy ....GFG CPLDQMQCHR HCQTITGRSG GYCSGPLKLT CTCYR.....
Lei qu ....GFG CPLNQGACHR HCRSIR.RRG GYCAGFFKQT CTCYRN....
Loop
α Helix
β Sheet
β Sheet

APPENDIX B

Pfam 7.8 (Saint Louis)
Home | Analyze a sequence | Browse Pfam | Keyword search | Taxonomy search | Swisspfam | Help

Starting search. Estimated time: 2 seconds (assuming all Wulfpack nodes are running). Please wait...

Pfam HMM search results, glocal+local alignments merged (Pfam_ls+Pfam_fs)

[Go here for an explanation of the format of the results]

	Model	Seq-from	Seq-to	HMM-from	HMM-to	Score	E-value	Alignment	Description
!!	Arthro_defensin	25	60	1	36	49.9	**4.8e-12**	glocal	Arthropod defensin

Alignments of top-scoring domains:

Format for alignment of query to Seed: Plain text

```
Arthro_defensin: domain 1 of 1, from 25 to 60: score 49.9, E = 4.8e-12
                  *->gkgcpvNhsaCaaHClakGGrrGGyCng.lkavCvCR<-*
                     g+gcp N   C++HC++++ rrGG C g+++++CvC+
       query    25    GFGCPFNAGKCHRHCKSIR-RRGGFCRGtFRTTCVCY    60
```

align query/25-60 to Arthro_defensin(ls) Seed

NEW! Phylogenomic analysis of query using RIO.

Given a query sequence, Pfam domain, and species, the RIO server will order sequences in the Pfam domain by orthology to the query. Many other options are available, and an annotated gene tree can be generated and viewed with ATV. The button below will send your query and Pfam domain hits to the RIO server.

send query to RIO

Home | Analyze a sequence | Browse Pfam | Keyword search | Taxonomy search | Swisspfam | Help
Comments, questions, flames? Email <pfam@genetics.wustl.edu>.

Arteri_glycop <-- --> Arv1

Arthro_defensin

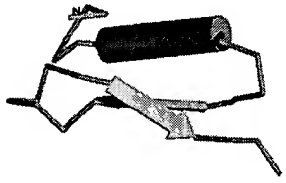


Figure 1: 1fjn
Antimicrobial protein
Solution structure and activity of the four disulfide bond mediterranean mussel defensin mgd-1

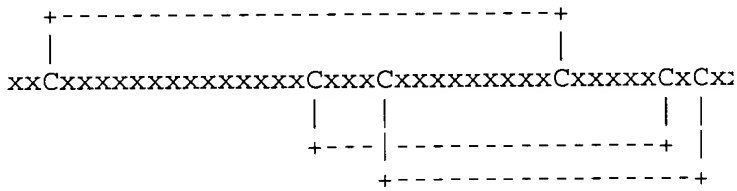
Image from PDBsum database

Accession number: **PF01097**
Arthropod defensin

Description

Arthropod defensins are a family of insect and scorpion cysteine-ric antibacterial peptides, primarily active against Gram-positive bacteria [MEDLINE:89098894], [MEDLINE:90293084], [MEDLINE:93228618], [MEDLINE:92105112], [MEDLINE:9304935(All these peptides range in length from 38 to 51 amino acids. There are six conserved cysteines all involved in intrachain disulfide bond

A schematic representation of peptides from the arthropod defensir family is shown below.



'C': conserved cysteine involved in a disulfide bonc

Although low level sequence similarities have been reported [MEDLINE:89098894] between the arthropod defensins and mammalian defensins, the topological arrangement of the disulfide bonds as well as the tertiary structure [MEDLINE:90382590] are completely different in the two families.

Description text from InterPro entry IPR001542

Sequence information

Alignment

Visualize domain

Species

Seed (15) Full (38)

Format: Hyperlinked plain text

Retrieve alignment

structures

Seed (15) Full (38)

display 10 per page.

Retrieve domain structures

distribution

Tree depth: all

View species tree

Literature References

[1]
Refined three-dimensional solution structure of insect defensin A.
Cornet B, Bonmatin JM, Hetru C, Hoffmann JA, Ptak M, Vovelle F;
Structure 1995;3:435-448.

Database References

HOMSTRAD	Arthro_defensin
PDB	1ica 1fjn
PROSITE	PDOC00356
SCOP	1ica (family)
INTERPRO	IPR001542

HMMER build information

	Pfam_ls [download HMM]	Pfam_fs [download HMM]
Gathering cutoff	25.00 25.00	25.00 25.00
Trusted cutoff	34.40 34.40	32.50 32.50
Noise cutoff	18.40 18.40	18.40 18.40
Build method of HMM	hmmbuild -F HMM_ls SEED hmmcalibrate --seed 0 HMM_ls	hmmbuild -f -F HMM_fs SEED hmmcalibrate --seed 0 HMM_fs

Pfam specific information

Author of entry	Finn RD, Bateman A
Type definition	Domain
Alignment method of seed	Clustalw
Source of seed members	Prosite

Help

Comments, questions, flames? Email <pfam@genetics.wustl.edu>.

APPENDIX C

Theoretical pI/Mw for the protein sequence MKSIAIIFIV ... TFRTTCVCYR :

Theoretical pI/Mw: 9.44 / 6830.19

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